

INFLUENCE OF INGESTED FOODS ON THE ORAL TOXICITY IN MICE OF CRYSTALLINE BOTULINAL TYPE A TOXIN¹

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Botulinal toxin is an oral poison. Yet it has been observed by a number of workers that proteolytic enzymes can destroy the toxicity of botulinal toxin (see review by Meyer and resistance by the toxin to the activity of proteolytic enzymes. Since botulinal toxin can be destroyed by proteolytic enzymes, and will be exposed to these enzymes in the alimentary tract,

TABLE 1

Effect of various foods on the oral toxicity in mice of type A crystalline botulinal toxin
(Data are the number of experiments giving the indicated result)

Food	Feeding Status of Mice before Ingestion of Food Tested					
	Starved Total deaths relative to control			Not starved Total deaths relative to control		
	Increased	No change	Decreased	Increased	No change	Decreased
Pellets.....	2					
Olive oil.....	6	2		2		
Olive oil-pellet....						
Mayonnaise.....				1	1	
Skim milk powder.....		1	1			3
Mayonnaise-skim milk powder....						2
Egg albumen.....	6					

In the experiments using commercial mouse pellet food and the olive oil-pellet mixture, mice starved for 24 hr were permitted to feed freely on the indicated food for 1 hr prior to receiving 0.25 ml doses of toxin.

Olive oil was used as such. Mayonnaise was diluted 1.5 times with water, skim milk powder was given as 10 to 66 per cent suspension in water, egg albumen as a 25 per cent suspension in water. The skim milk-mayonnaise mixture contained 5 g of skim milk powder and 5 g of mayonnaise per 15 ml of added water.

In some cases the animals were permitted to feed freely, whereas in others 0.25 ml *per os* injections of the indicated food were given. In some experiments a sham injection was given to control mice not receiving the food. This was a control measure, since mice given food by *per os* injection were subject to two separate injections, one of food and one of toxin. Sham injections did not seem to affect results and can be considered noninjurious. Autopsy of more than 80 mice revealed no evidence of perforation or signs of local hemorrhage along the route of injection.

Lamanna, 1959). Evidently the capacity to act as an oral poison cannot be a matter of absolute

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it is pertinent to ask how the toxin can act effectively as an oral poison. Complete understanding of this problem requires knowledge of the effects of ingested foods on toxicity, since in clinical cases of food poisoning toxin is swallowed with a variety of different foods. It is conceivable that ingested foods might affect the oral potency of

TABLE 2

Effect of natural feeding of mice on pellets before per os injection of toxin

Cumulative Deaths after Receiving Toxin							
Toxin dilution	Hr	Pellet fed	Not fed	Toxin dilution	Hr	Pellet fed	Not fed
1:10	12	4	0	1:40	12	0	0
	18	9	1		18	0	0
	24	15	1		24	0	0
	36	17	4		36	5	1
	48	18	6		48	6	2
	>48	18	9		>48	11	3
1:20	12	0	0	1:80	12	0	0
	18	3	0		18	0	0
	24	9	1		24	1	0
	36	11	2		36	1	1
	48	15	4		48	2	1
	>48	19	7		>48	5	1
Total deaths						53	20

Mice were not fed for 24 hr. Then the fed groups were given free access to pellets for 1 hr, after which time the food was removed and toxin injected *per os* 15 min later. The mice were not permitted to feed again for another 6 hr. No attempt was made to determine the amount of food consumed per mouse. The non-fed mice were not fed for 24 hr and were also starved the additional 6 hr after receiving the toxin. Twenty mice were injected per group.

toxin by two mechanisms acting alone or together: by influencing the rates of intestinal detoxification processes including proteolysis, and by modifying the permeability of the intestinal tract for the toxin. For lethality it is only necessary for the minimal fatal dose of toxin to escape across the alimentary tract barriers before detoxification has had time to proceed to completion. The present investigation has sought to observe the effects of ingested foods on the oral toxicity of botulinal toxin. May and Whaler (1958) have reported oral toxicity to be increased for starved mice permitted to feed.

MATERIALS AND METHODS

Toxin. Type A crystalline toxin, generously supplied by Dr. Edward J. Schantz of Fort Detrick was employed. In different titrations between 50,000 to 250,000 intraperitoneal LD₅₀ proved equivalent to 1 LD₅₀ *per os*.

Diluent. Toxin was stored and diluted for use in buffer solutions containing 0.2 per cent gelatin. Citrate buffers in the range of pH 3 to 5 were employed for storing stock solutions. For diluent, a phosphate buffer, pH 6.3 to 6.5, was employed.

Treatment of mice. Namru strain 22 to 30 g white mice were studied. Within a single experiment, mice were selected at random from the same population pool. To reduce the chance for the order of injection affecting results rather than the variable under test, the sequences of

TABLE 3

Effect of natural feeding of mice on pellets alone, olive oil alone, and a pellet-olive oil mixture (1:1 by weight) on oral toxicity of botulinal toxin

Cumulative Deaths after Receiving Toxin <i>per Os</i>											
Toxin dilution	Hr	Pellet	Pellet and olive oil	Olive oil	Not fed	Toxin dilution	Hr	Pellet	Pellet and olive oil	Olive oil	Not fed
1:10	12	6	9	9	6	1:40	12	6	2	4	3
	24	12	12	13	13		24	13	7	7	5
	36	16	15	16	16		36	17	11	9	5
	48	17	16	19	16		48	17	11	10	6
	>48	20	18	20	20		>48	18	15	12	10
1:20	12	8	5	6	1	1:80	12	3	2	0	1
	24	14	11	11	4		24	5	2	0	2
	36	17	12	15	8		36	10	2	1	3
	48	17	13	15	8		48	11	2	2	4
	>48	19	14	16	11		>48	14	4	3	6
Total deaths.....								71	51	51	47

The procedure for this experiment was the same as for table 2.

TABLE 4

Effect of per os injection of egg albumen and olive oil on the toxicity of botulinal toxin

Cumulative Deaths after Receiving Toxin <i>per Os</i>									
Toxin dilution	Hr	Sham	Albumen	Olive oil	Toxin dilution	Hr	Sham	Albumen	Olive oil
1:2	12	1	6	9	1:8	12	2	4	3
	24	7	16	15		24	6	9	8
	36	10	20	18		36	7	14	12
	48	12	20	20		48	9	15	13
	>48	17	20	20		>48	11	20	16
1:4	12	0	5	3	1:16	12	2	1	0
	24	1	12	6		24	3	1	4
	36	2	14	9		36	3	2	6
	48	4	16	10		48	4	2	6
	>48	9	18	14		>48	5	12	6
Total Deaths.....							42	70	56

Mice were starved 12 hr. Then control mice were given a sham *per os* injection; 0.25 ml of 25 per cent egg albumen was given to a second group, and 0.25 ml of olive oil to a third group. Toxin, 0.25 ml, was then given to all mice. Mice were starved an additional 6 hr after receiving toxin before being permitted to feed freely on laboratory pellet chow. Each group consisted of 20 mice.

TABLE 5

Effect of differences in the sequence of per os administration of olive oil and botulinal toxin on the death time

Hr	Cumulative Deaths after Toxin Ingestion				
	Toxin alone	Toxin immediately followed by olive oil	Toxin followed 1 hr later by olive oil	Olive oil followed 1 hr later by toxin	Olive oil followed immediately by toxin
12	5	5	2	9	6
24	18	19	21	34	28
36	26	36	40	44	40
48	38	46	46	47	45
60	42	47	47		
72		48	48		
>72	45	49		48	48

A total of 50 mice were injected in each group.

injections of different groups of mice were randomized. A slightly curved, blunt bulbous tipped 18 gauge, 3 cm long needle was used for oral injections. Unless stated otherwise, these injections were done with 0.25 ml quantities of reagents. The subjects tolerated without regurgitation a single dose *per os* of 0.75 ml. Foods and toxin were given in separate exposures. This was done in preference to mixing toxin and foods *in vitro*

to insure that all results were the consequence of interactions *in vivo* exclusively. At all times the mice had free access to drinking water, even when access to food was subject to control. Since deaths from botulism in mice are rare after 5 days, the animals were observed for this length of time after receiving toxin.

RESULTS AND DISCUSSION

Experiments have been summarized in table 1. In substance they indicate a tendency for both fatty and proteinaceous foods to increase the potency of orally administered crystalline toxin. The increase in potency was brought to notice by observations of increases in total numbers of deaths, and decreased death times among animals receiving the food relative to controls not receiving the food. The exception was skim milk powder which gave fewer deaths with toxin relative to control exposed animals. This action was of a low order, decreases of LD₅₀ being less than 2-fold. Variation of results in replicate experiments does not make it possible presently to precisely state quantitatively the enhancement effects of foods except to say that increased potency was of the order of less than 5-fold increases in LD₅₀ values.

Some of the experiments are recorded in detail

TABLE 6
Effect of brandy (96 proof) on the per os toxicity of botulinal toxin in the presence of food
 (a) Presence of olive oil*

Hr	Cumulative Deaths after Ingestion of Toxin								
	Undiluted			1:2			1:4		
	Control	Olive oil	Olive oil and brandy	Control	Olive oil	Olive oil and brandy	Control	Olive oil	Olive oil and brandy
6	2	4	3	2	0	3	1	4	1
12	5	15	8	3	1	4	3	4	2
18	12	17	12	4	4	5	5	4	2
24	15	19	12	9	9	9	6	6	3
30	18	20	16	12	12	10	8	10	7
36	19		19	15	12	11	9	12	8
42	19		20	16	12	12	9	14	10
48	20			16	13	13	10	15	13
60				17	15	14	11	15	14
72				18	15	14	11	15	15
>72					16	15	13	17	17
Total deaths.....							51	53	52

(b) Presence of egg albumen†

Hr	Cumulative Deaths at Hr Indicated after Toxin Ingestion								
	Dilution of toxin								
	1:1.5			1:2			1:4		
	Sham	Albumen	Albumen and brandy	Sham	Albumen	Albumen and brandy	Sham	Albumen	Albumen and brandy
12	1	3	1	1	2	0	1	1	0
24	2	6	6	2	2	2	1	2	2
36	2	12	8	3	5	2	2	3	3
48	4	14	10	3	5	3	2	4	4
60	5	15	10	3	7	4	2	4	4
72	5	15	10	4	7	4	2	4	4
>72	5	15	13	4	9	5	2	4	4
Total deaths.....							11	28	22

Presence of egg albumen (continued)‡

12	3	1	4	4	3	5	0	1	2
24	3	5	4	5	6	7	3	3	3
36	6	8	8	6	11	7	4	5	3
48	7	12	9	8	14	9	5	5	4
60	8	14	10	9	16	10	6	5	4
72	8	14	11	11	16	10	6	5	4
>72	9	14	11	11	16	10	7	6	4
Total deaths.....							27	36	25

* Twenty mice injected per group. Mice were starved 12 hr, then divided into 3 groups for the treatments. Control groups received 0.25 ml of saline; olive oil group 0.25 ml; olive oil-brandy groups received 0.25 ml of each substance. Toxin, 0.25 ml, was given *per os* 15 min after injection of food.

† Twenty mice were injected per group. The control group received a sham injection *per os*; the group receiving albumen received 0.25 ml of a 25 per cent water suspension; the group receiving brandy received 0.25 ml of a 50:50 mixture of brandy with 25 per cent albumen suspension. Injection *per os* with 0.25 ml toxin followed after the injection of test material.

‡ Twenty mice were injected per group. The control group received 2 sham injections followed by the toxin 15 min later. The groups receiving albumen received 0.25 ml of albumen suspension, then 0.25 ml of tap water and the toxin 15 min later. The groups receiving brandy were first given 0.25 ml of albumen, then 0.25 ml of brandy, and then 15 min later were given 0.25 ml of toxin solution.

The toxic dose of brandy for the mice is greater than 0.25 ml of undiluted brandy. A dose of 0.75 ml is invariably lethal. The experiments with brandy were, therefore, limited to 0.25 ml and lesser quantities.

for commercially prepared mouse rations (tables 2 and 3), olive oil (tables 3-5), and egg albumen (tables 4 and 6). It is our impression that two foods, each alone capable of increasing toxicity, do not have an additive effect in mixtures (for example, table 2).

In giving toxin and a food, the sequence of the injections can be varied. In general, it was found that toxicity was more likely to be enhanced if the ingestion of toxin followed the food rather than *vice versa*. An experience of this sort is recorded in table 5 in which a single lethal dose of toxin gave reduced death times if it followed rather than preceded the administration of olive oil *per os*.

Since dissimilar foods affect oral toxicity, it is not possible from the data at hand to defend any single mechanism for explaining all the results. Probably different mechanisms operate when unrelated foods as egg albumen and olive oil each alone exhibit the same qualitative enhancement effect, and yet do not act additively in mixtures. One conclusion is clear. Quantitative statements of the oral potency of botulinal toxin for the purpose of comparison require knowledge of the kinds of foods consumed.

It is a part of the mythology of botulism that those who partake of alcoholic beverages at the fatal feast suffer less serious consequences than those who did not indulge. A few experiments, therefore, have been performed to test this belief. As can be seen in table 6, ingestion of mixtures of brandy and egg albumen, a reasonable simulation of human experience, and brandy with olive oil tended only to reverse the enhancing effect on toxicity of the food without reducing potency below the level experienced upon in-

gestion of toxin alone. Thus, the consumption of alcoholic beverages has no remarkable prophylactic value in botulism except insofar as the true imbibitor eats less food. Taking into account the weight difference between man and mouse at higher doses of brandy than those employed in the experiments of table 6, one is subject to the risk of drinking lethal quantities of brandy. In such an event the anticipated cure might be more pleasant than the disease but equally fatal.

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SUMMARY

The potency of orally administered crystalline type A botulinal toxin is influenced by ingested foods. The tendency is for commercial mouse pellet feed, egg albumen and olive oil to increase toxic potency, and skim milk powder to decrease potency. During a meal, brandy would have to be consumed in copious quantity to effect significant decreases in oral toxicity. Quantitative statements of oral toxicity, to be most meaningful, should include information on the state of food consumption by test animals.

REFERENCE

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